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Previews

Tolerance Induction by Allogeneic Hematopoietic Stem Cells

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In this issue of *Cell Stem Cell*, Zheng et al. (2011) report that HSCs expressing PD-L1 display enhanced engraftment in irradiated allogeneic recipients. Independently in *Nature*, Fujisaki et al. (2011) observe allogeneic HSCs persisting in proximity to regulatory T cells in nonirradiated recipients, further connecting HSCs and immune tolerance.

Allogeneic transplantation of HSCs is a potential therapy for a number of nonmalignant or malignant disorders for which the replacement of diseased HSCs with new ones that possess normal myeloid and lymphoid reconstitution potential is desirable. Allogeneic HSC engraftment can be achieved across major donor-host genetic disparities using potent conditioning regimens, but at the cost of (1) high treatment-related mortality due to graft-versus-host-disease (GVHD), a condition caused by donor-derived T lymphocytes, and (2) infections, which are precipitated by the immunosuppressive treatments needed to treat GVHD and prevent graft rejection. Transplantation using reduced intensity conditioning and T cell depleted grafts is better tolerated, but the balance between engraftment and rejection continues to pose a significant challenge (reviewed in Welniak et al., 2007).

In murine models, purified HSCs (which lack the potential to induce GVHD) can establish long-term hematopoietic chimerism, but only in conjunction with significant host conditioning. Even when using total body irradiation, substantially larger doses (10- to 60-fold greater) of allogeneic HSCs are needed to achieve radioprotection and hematopoietic reconstitution in comparison with syngeneic HSCs (Shizuru et al., 1996). In addition, Reisner and colleagues demonstrated in 1995 that the HSC dose required to induce chimerism further increases as the irradiation dose decreases (Welniak et al., 2007). In this issue of *Cell Stem Cell*, Zheng et al. (2011) generated HSCs with increased allogeneic engraftment ability. They compared the engraftment potential of $Lin^-Sca\text{-}1^+Kit^+CD34^-Flk2^-$ bone marrow cells cultured 8 days in a previously defined serum-free medium to that of ex vivo purified HSCs isolated from C57BL/6 donors and infused in lethally irradiated BALB/c recipients. Based on limiting dilution studies in the presence of admixed syngeneic competitor cells, they calculated that ex vivo expanded HSCs contained 11 times more engrafting cells than the original input cell number, but engrafted 40-fold better than their purified noncultured counterparts, as determined by hematopoietic reconstitution of different blood lineages, 16 weeks posttransplant. This suggested that a factor independent of the cell number contributed to the persistence of the infused HSCs. Zheng and colleagues demonstrated that this factor was an elevated expression of PD-L1, a T cell inhibitory molecule, by the HSCs themselves. Only the PD-L1-positive fraction of HSCs displayed enhanced repopulating capacity in allogeneic settings. When the in vitro expanded HSCs were generated from PD-L1 KO donors, their engraftment ability was decreased or abolished, depending on the number of cells infused, in comparison with that of wild-type equivalents.

B7-H1/PD-L1 is a transmembrane protein that belongs to the B7 family of T cell costimulatory molecules. The binding of PD-L1 to its receptor PD-1 dampens T cell activation, reduces IL-2 and IFNγ secretion, decreases proliferation and cytotoxicity, and induces apoptosis (reviewed in Francisco et al., 2010). PD-L1 is expressed by immune cells such as macrophages, dendritic cells, and T and B cells, but is also expressed in other tissues including endothelial cells and pancreatic islet cells. Studies in mouse models demonstrate that the PD-L1/PD-1 pathway induces T cell exhaustion in chronic viral diseases but also contributes to the maintenance of peripheral tolerance against self-antigens and to the protection of tissues from inflammation during infections (Mueller et al., 2010). In humans, there is increasing evidence that some tumor cells express PD-L1, which serves as an immune escape mechanism (Dong et al., 2002).

Zheng et al. showed that the cultured PD-L1-expressing HSCs inhibit the proliferation of allogeneic T cells in vitro, whereas PD-L1 KO HSCs do not. They also showed, using immunocompromised SCID/BALB/c recipients, that T cells, but not NK cells, were the immune cells targeted by HSC-induced tolerance. The authors verified that cultured human cord blood HSCs express PD-L1 and reduce allogeneic T cell proliferation in mixed leukocyte cultures.

A recent study investigating the fate of allogeneic HSCs in nonirradiated immunocompetent mice points to the immune privilege of HSCs, albeit in a very distinct fashion, involving the topology of the HSC niche and its proximity to regulatory T cells. Fujisaki and colleagues (Fujisaki et al., 2011) performed allogeneic transplantation of $c\text{-}kit^+Sca^+Lin^-$ bone marrow cells from C57BL/6 donors to BALB/c recipients, without any conditioning, and followed their persistence in recipients' bone marrow. Using intravital microscopy to track single cells, they detected 90% of the infused enriched HSCs in the vicinity of the endosteal surface, 1 month after transplantation, indicating that the

transplanted cells were not rejected by the recipient's intact immune system. The persisting quiescent HSCs did not contribute to hematopoiesis, but at least a subset of them maintained long-term repopulating capacity, as confirmed by secondary transplantation. The authors also established that this immune tolerance was specific to HSCs, because coinjected Lin^+ bone marrow cells disappeared 1 week after infusion. The authors observed that bone marrow $FoxP3^+$ Tregs were localized near the HSC niche and surrounded infused HSCs in the allogeneic transplantation experiments. These Tregs expressed TGFβ and increased levels of IL-10 following successful allogeneic HSC seeding. Treg depletion by either diptheria toxin or anti-CD25 antibody led to near full rejection of the previously seeded HSCs, which was associated with a local elevation of TNF-α and IL-4.

These two studies reveal new facets of the immunobiology of allogeneic HSC engraftment. Zheng et al. show engraftment of cultured HSCs overexpressing PD-L1 in mice in which the medullary environment has been disrupted by irradiation, resulting in full hematopoietic reconstitution. Fujisaki et al. show stable seeding of allogeneic HSCs in the subendosteal medulla and maintenance of HSC function without active hematopoietic activity in nonconditioned recipients. As different as they are, there are some points of convergence between the two reports.

Recently, Sharpe and colleagues demonstrated that PD-L1 inhibits effector T cell functions but can also convert naive CD4 T cells into induced Tregs in the presence of TGFβ (reviewed in Francisco et al., 2010). IL-10, on the other hand, has been shown to increase PD-L1 expression by monocytes in another immunosuppressive milieu, the tumor microenvironment (Kuang et al., 2009). The impact of irradiation on medullary architecture and function is overwhelming and likely affects all of the above parameters. It is important to note, however, that radiosensitivity differs between cell types. Thus, Tregs are relatively more resistant than other T cell subsets (Nador et al., 2010), which may favor the regeneration of an immunoprotective niche soon after HSC infusion in irradiated recipients. The relationship between PD-L1 expression in HSCs and their progeny, Tregs and the stem cell niche, thus warrants further exploration, as does their potential interplay with mesenchymal stem cells, which are also immunosuppressive under the right circumstances (Caplan and Correa, 2011).

The persistence of quiescent allogeneic HSCs in nonirradiated recipients is intriguing, but is per se of no clinical benefit. Having stealthily taken root within the host organism, the foreign HSCs would have to extend tolerance induction to their progeny in order to productively contribute to hematopoiesis. PD-L1, which can protect tumors (Dong et al., 2002) and parenchymal tissues against immune assaults (Mueller et al., 2010), may find a role beyond that shown here in incoming HSCs. PD-L1 may perhaps be harnessed to afford immunoprotection to the progeny of HSCs, or the progeny of other stem cells as well (Zhao et al., 2011). Interestingly, Zheng et al. found PD-L1 to be upregulated by cultured human cord blood $CD34^+$ cells, which may be exploited in the clinic in the context of single- or double-cord blood transplantation, a promising therapeutic strategy that may be compromised by insufficient cell dose or immune rejection.

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